Malolactic Activity of Lactic Acid Bacteria during Sauerkraut Fermentation

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ABSTRACT: The frequency of lactic acid bacteria (LAB) involved in sauerkraut fermentation with (MDC$^+$) or without (MDC$^-$) the ability to decarboxylate malic acid was determined. The MDC$^+$ phenotype was found in > 99% of homofermentative LAB isolated from commercial fermentations. In contrast, heterofermentative LAB isolates from 0.25, 3, 7, and 10 d had only 53%, 54%, 15%, and 11% MDC$^+$ phenotype, respectively, indicating that more than 1 strain or species was involved. The malolactic reaction was demonstrated in cabbage juice with known strains of *Leuconostoc mesenteroides*, raising the question of desirability of such activity in cultures selected for the controlled fermentation of cabbage.

Keywords: malolactic activity, *Leuconostoc mesenteroides*, sauerkraut, fermentation, lactic acid bacteria

Introduction

Heterofermentative lactic acid bacteria (LAB), primarily *Leuconostoc mesenteroides*, have been reported to predominate the early stage of sauerkraut fermentation. This stage largely dictates the production of flavor volatiles and balance between lactic and acetic acids (Pederson and Albury 1969). The commercial fermentation of sauerkraut results from resident LAB on cut and salted cabbage and therefore has the potential for high variability in quality because of variability of the naturally occurring microflora. Environmental conditions such as salt concentration and temperature are also variable at times and affect the growth and competitive situation of the naturally present microorganisms. To minimize these sources of variation and produce a more consistent product, starter cultures have been proposed for sauerkraut (Fleming and McFeeters 1981; Fleming 1987; Harris and others 1992).

In screening *L. mesenteroides* cultures in our laboratory for use in controlled fermentations, it has been observed that some have the ability to decarboxylate L-malic acid (MDC$^+$) to L-lactic acid and CO_2 with a malolactic enzyme (Kunkee 1967; Radler 1986) and others do not (MDC$^-$). The reaction involves the uptake of protons in an equimolar concentration, which may have some influence on pH. The malolactic reaction has been found to be important in some wine and cucumber fermentations where malic acid is naturally present. Certain wines benefit from the biological de-acidification that secondary malolactic fermentation provides, resulting in a less acidic wine with distinctive flavors (Henick-Kling 1995). However, in cucumber fermentation the malolactic reaction is undesirable because of carbon dioxide production, which contributes to bloater formation, a defect in pickles (Fleming and others 1973; Fleming and Pharr 1980; McFeeters and others 1984). Malic acid (12.2 ± 1.6 m*M*) has been reported in raw cabbage (Fleming and others 1988) with a concentration range between 5 to 25 m*M* for different cultivars and batches of cabbage (unpublished data). Because malic acid is a natural component of cabbage, there is potential for its decarboxylation to take place during sauerkraut production.

Malolactic activity of LAB may be important in sauerkraut fermentation for at least 2 reasons. First, conversion of malic acid to lactic acid early in the fermentation, before significant sugar metabolism, has potential for influencing the rate of pH decrease, with possible influences on relative growth of various bacterial species and on chemical changes that lead to flavor development. Secondly, additional CO_2 production during the early stage of fermentation could exacerbate the heaving problem associated with commercial sauerkraut fermentation. Heaving has been described as the increase in sauerkraut volume because of rapid CO_2 production by heterofermentative LAB, resulting in gas entrapment within the sauerkraut and a rise in brine level in the tank (Fleming and others 1988). The objectives of this research were to determine the frequency of MDC$^+$ and MDC$^-$ heterofermentative LAB among our culture collection and during the fermentation of cabbage to sauerkraut, and to assess the significance of the malolactic reaction on fermentation chemistry.

Materials and Methods

LAB

LAB isolates were obtained from the USDA-ARS culture collection, laboratory fermentations, and commercial fermentations. Sixteen previously identified strains of *L. mesenteroides* from the USDA-ARS Food Fermentation Laboratory (FFL, located in the Food Science Dept., North Carolina State Univ., Raleigh, N.C., U.S.A.) culture collection were cultivated in de Man, Rogosa, Sharpe (MRS) medium and tested in malolactic differential (MD) medium (Daeschel and others 1984) to determine malolactic phenotype. *Lactobacillus plantarum* BI 7 (MOP 3) and MU 45 (MOP3 M6, Daeschel and others 1984) were used as MDC$^+$ and MDC$^-$ controls, respectively.

Enumeration and isolation of LAB

Brine samples were spiral-plated (Autoplate 4000, Spiral Biotech, Norwood, Mass., U.S.A.) on modified MRS agar (0.02% azide) and incubated at 30 °C for 48 h. After plate counts were obtained, randomly selected isolated colonies were picked with sterile toothpicks

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into 96-well microtiter plates containing MRS broth. Inoculated MRS broths were incubated at 30 °C for 18 h to prepare overnight cultures for further testing.

Malolactic activity

Overnight cultures were diluted 1:100 in 0.85% saline, inoculated into MD medium (Daeschel and others 1984), and incubated at 30 °C for 48 h. Turbidity and color changes were recorded after 48 h of incubation. MDC$^+$ cultures resulted in turbid broths that exhibited no color change. MDC$^-$ cultures changed the color of the broth from clear dark blue to turbid green. No turbidity was interpreted as no growth. HPLC analysis of fermented MD medium for malic acid concentration was used to verify results.

Gas production

Overnight cultures of LAB were inoculated into 10 mL MRS broth containing inverted glass tubes (approximately 6 × 50 mm) and incubated at 30 °C. Results were recorded after 48 h. Cultures producing gas within the 48-h incubation period were presumed to be heterofermentative LAB. Those cultures that did not produce gas within 48 h were presumed to be homofermentative LAB.

Fermentation chemistry

Fermentation products and malic acid concentration were determined by a Dionex HPLC system (Dionex Corp., Sunnyvale, Calif., U.S.A.), PLHi-Plex H column using 3 m*M* heptafluorobutyric acid as eluent and a conductivity cell for detection (McFeeters 1993).

Fermentations

Laboratory fermentations with Cecile, Hinova, Carlton, and Atria cultivars of kraut cabbage were conducted in 16-oz jars at a 60:40 cabbage-to-brine ratio with 2% NaCl (equilibrated). Duplicate jars for each treatment were incubated at 18 °C, the optimum temperature for sauerkraut fermentation as determined by Parmele and others (1927). Brine samples were taken aseptically through a septum in the lid of each jar after 3.5 d of fermentation. LAB growth was monitored by enumeration on modified MRS agar (colony-forming units [CFU]/mL), and 46 LAB isolates from each jar were tested for malolactic activity in MD medium. Chemical changes were monitored by measurement of pH and fermentation end products. Three 90-ton commercial sauerkraut fermentations tanks loaded on the same day were sampled. Brine samples (100 mL) were taken from 2 locations diagonally across the rectangular tanks at 6 h and 3, 7, and 10 d of fermentation. The brine was sampled through a 1-cm-dia stainless-steel tube inserted to a depth of about 60 cm from the top and 60 cm from the side of the tank and allowed to fill through a hole in the bottom of the tube. The brine samples were transferred to sterile plastic tubes, cooled on ice, and immediately shipped to our laboratory by overnight mail. LAB growth was monitored by enumeration on modified MRS agar (CFU/mL). Forty-five LAB isolates from each commercial brine sample were tested for malolactic activity in MD medium. Ten LAB isolates from each sample were also tested for gas production to determine relative proportions of heterofermentors and homofermentors. Chemical changes were monitored by measurement of pH, titratable acidity (TA), NaCl, fermentation products, and residual sugars of each sample (Fleming and others 2001).

Culture identification

Selected LAB isolates from the USDA-ARS FFL culture collection, laboratory fermentations, and commercial fermentations were identified using BIOLOG AN MicroPlate™ (Biolog, Inc., Hayward, Calif., U.S.A.) according to the manufacturer's instructions. Identifications were made using the Biolog Micrologl 4.01B database. Strains for identification from commercial fermentations were chosen from isolates that exhibited differentiating characteristics, such as gas ±, MDC ±, at each time point. They were not chosen to reflect the majority of what was present in the sauerkraut at that time point, but rather the diversity of what was present.

Cabbage juice

Cabbage juice was prepared from locally purchased cabbage of unknown cultivar. Outer leaves and cores were removed, and the remaining cabbage was quartered and heated in the autoclave for 10 min at 121 °C, bringing the internal temperature of the cabbage to 95 °C to inhibit enzymes that catalyze production of inhibitory substances in cabbage juice (Kyung and Fleming 1994; Kyung and others 1997). Heated cabbage pieces were processed with a Braun Juicer (Braun, Kronberg, Germany). The resulting juice/slurry was centrifuged to remove particulates. NaCl (2%) was added, and the resulting product was sterile-filtered (Corning 0.22-μm cellulose acetate bottle-top filter, Corning, New York, N.Y., U.S.A.). Cabbage juice was tested for its ability to support growth of LAB before its use in these experiments. Excess cabbage juice was stored at –20 °C. Natural sauerkraut microflora were obtained by setting up duplicate natural cabbage fermentations in 16-oz glass jars. Cabbage was shredded and salted (2% NaCl), packed into 16-oz glass jars, and incubated at 18 °C. A 1-mL brine sample was removed from each jar after 2 d. Each brine sample was centrifuged to obtain a cell pellet, which was then resuspended in 1 mL of 0.85% saline (approx. 10^8 LAB/mL) for use as an inoculum into cabbage juice. Selected strains from the USDA-ARS FFL culture collection were precultured in MRS and cells were resuspended in 0.85% saline before inoculation into cabbage juice. Fermentation with selected strains from the USDA-ARS FFL culture collection and natural sauerkraut microflora was monitored in sterile-filtered cabbage juice inoculated with approximately 10^6 CFU/mL of each culture tested. Fermentations were conducted at 18 °C and sampled at 7 h, and 1, 4, 11, and 30 d. Each sample was analyzed for LAB (CFU/mL), pH, malic acid concentration, sugars, and fermentation end products.

Results and Discussion

Of the 16 strains of *L. mesenteroides* from the USDA FFL culture collection, 10 were MDC$^+$, and 6 were MDC$^-$. Chen and others (1983) showed that 3 strains of *L. mesenteroides* did not exhibit malolactic activity during fermentation of green bean juice. On the contrary, other researchers (Schutz and Radler 1974; Winter and Kandler 1977; Garvie 1984) reported *L. mesenteroides* strains that were MDC$^+$. The current research confirms that malolactic activity in *L. mesenteroides* is strain-dependent. Other heterofermentative LAB, *Lactobacillus brevis* LA 36, *Lactobacillus cellobiosis* LA 31, and *Leuconostoc paramesenteroides* LA 225, were MDC$^+$, confirming previous results (Chen and others 1983).

Growth of selected LAB strains in cabbage juice (2% NaCl, 18 °C) demonstrated the ability or inability of MDC$^+$ and MDC$^-$ strains to carry out the malolactic reaction in a model sauerkraut fermentation. The MDC$^+$ *L. mesenteroides* ATCC type strain, LA 81, and natural sauerkraut microflora, consumed all of the malic acid in cabbage juice within 1 d of fermentation (Figure 1a). The MDC$^+$ *Weissella confusa* ATCC type strain, LA 109, also quickly depleted the malic acid in cabbage juice (data not shown). LA 10 and LA 12, which are MDC$^-$ *L. mesenteroides* strains, consumed no malic acid even after 30 d, so that approximately 6 m*M* malic acid remained in the fermented juices. Strains LA 81 (MDC$^+$) and LA 12 (MDC$^-$) both produced heterofermentative end-products (Figure 1b and 1c) and had the same final pH of 3.6 (Figure 1d). This was similar to the final

pH of 3.8 reported for *L. mesenteroides* grown in cucumber juice without NaCl (McDonald and others 1990). It is also reasonable for the pH to be slightly lower in the cabbage juice because it contained NaCl, which is known to depress pH. After 7 h of fermentation, the pH of the LA 81 (MDC+) fermented cabbage juice was 0.1 units higher than the LA 12 (MDC–) fermented juice ($P < 0.05$), but after 1 d, the pHs were almost identical (Figure 1d). Evidently, the pH lowering due to production of lactic and acetic acids (approximately 80 m*M* total) during fermentation outweighed the slight deacidification of the cabbage juice from the conversion of 6 m*M* malic acid to lactic acid. These results demonstrated the conversion of malic acid to lactic acid in cabbage juice by LAB strains with malolactic activity as well as natural sauerkraut microflora. Despite the deacidifying nature of the malolactic reaction, there was no effect on the final pH of the cabbage juice. However, sauerkraut fermentation takes place at a much slower rate than the fermentation of cabbage juice inoculated with high numbers of LAB. Because the malolactic reaction took place faster than the conversion of sugars to acids, the malolactic reaction may play some role early in the fermentation or when cabbage cultivars higher in malic acid concentration are used. The fermentation of cabbage juice by natural sauerkraut microflora produced less acetic acid than the *L. mesenteroides* strains tested, leading to a more gradual decline in pH (Figure 1d). Interestingly, the production of lactic and acetic acids and the rate of pH decline in the cabbage juice inoculated with natural sauerkraut microflora were very similar to that of the cabbage juice fermented with *W. confusa* LA 109 (data not shown), which had a final pH of 4.0.

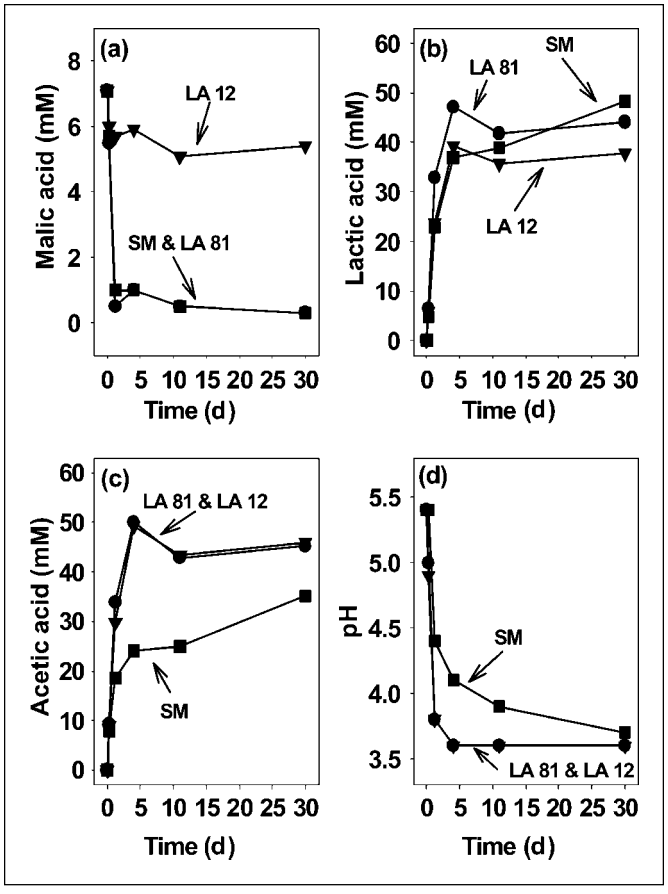

Figure 1—Fermentation of cabbage juice by MDC+ and MDC– *Leuconostoc mesenteroides* strains compared with natural sauerkraut microflora. ● = *L. mesenteroides* LA 81 (ATCC 8293, MDC+); ▼ = *L. mesenteroides* LA 12 (ATCC 23368, MDC–); ■ = natural sauerkraut microflora (SM).

Table 1—Brined cabbage fermentations[a] (2% NaCl, 18 °C)

Cabbage source					
Cultivar	Company	Fermentation	pH	LAB[b] (log CFU[c]/mL)	MDC+ (%)[d]
Cecile	A	1	5.76	3.65	11
Cecile	A	2	4.31	5.98	2
Hinova	A	1	5.75	5.98	13
Hinova	A	2	4.48	7.69	100
Cecile	B	1	5.26	7.70	0
Cecile	B	2	4.70	7.97	0
Carlton	C	1	4.68	7.74	100
Carlton	C	2	4.43	7.25	13
Atria	D	1	4.91	6.64	18
Atria	D	2	4.28	6.62	0

[a]Measurements taken after 3.5 d of fermentation.
[b]LAB = lactic acid bacteria.
[c]CFU = colony-forming unit.
[d]% MDC+ = number of isolates with malolactic activity/46 bacterial isolates tested × 100.

Laboratory sauerkraut fermentations by naturally present microflora on the cabbage showed that the percentage of MDC+ LAB after 3.5 d of fermentation was highly variable among sources (4 commercial firms, 3 states, 4 cultivars), as well as between duplicate fermentation jars (Table 1). Despite the controlled temperature and salt conditions, pH values and total LAB (CFU/mL) varied between duplicate fermentations, suggesting the possible benefit of a more controlled fermentation via starter culture incorporation. Cabbage fermentations that rely solely on the natural microflora of the cabbage are often variable in the rate at which they ferment. This particular batch of cabbage was also low in initial LAB count. Gardner and others (2001) have investigated the use of commercially available silage inoculants for mixed vegetable fermentations, and they proposed a mixed culture consisting of *L. plantarum*, *Pediococcus acidilactici*, and *L. mesenteroides* for fermenting carrot, cabbage, beet, and onion mixtures.

In commercial sauerkraut fermentations, homofermentative LAB were initially (6 h after tank packing) the predominant microorganisms, but the heterofermentative LAB quickly took over the fermentation and constituted 90% of the LAB population after 3 d ($P < 0.05$). At 7 d into fermentation, the heterofermentative LAB constituted approximately 60% of the LAB population and after 10 d, the homofermentors were already taking over the fermentation ($P < 0.05$). Overall, the total number of LAB increased between initial packing and 10 d of fermentation (Figure 2). This is typical of sauerkraut fermentation, as described by Fleming and others (1988). However, there are conflicting reports about which types of LAB are initially prevalent before fermentation begins. According to Mundt (1970) and Mundt and others (1967), *Leuconostoc* spp. are the predominant LAB isolated from vegetables. Teuber (1993) stated that 80% of LAB found on plant leaves are leuconostocs. However, other research (Dellaglio and others 1995) indicated that leuconostocs make up only a small percentage of the mesophilic microflora in their natural habitats, which are dominated mostly by homofermentative LAB. In the current study, homofermentative LAB predominated in freshly sliced and salted cabbage in commercial sauerkraut tanks, 6 h after packing. It is known that homofermentative LAB terminate the sauerkraut fermentation (Pederson and Albury 1969), so it is possible that residual homofermentative LAB were present in the sauerkraut tanks from previous fermenta-

tions. Pederson and Albury (1969) noted that poorly cleaned kraut vats allowed the homofermentative *L. plantarum* to remain on the tank walls and dominate the fermentation in areas close to the periphery of the vat. But in light of modern-day sanitation practices, it is more likely that the population of LAB on this sampling of kraut cabbage was naturally higher in homofermentative microorganisms.

Greater than 99% of the homofermentative LAB isolated from commercial fermentations during the first 10 d were MDC$^+$. However, the proportion of heterofermentative LAB exhibiting malolactic activity decreased ($P < 0.001$) during this time (Figure 3). Isolates taken after 6 h and 3, 7, and 10 d of fermentation contained 53%, 54%, 15%, and 11% MDC$^+$ heterofermentors, respectively. These data reflect a shift in the population sometime between 3 and 7 d of fermentation, at which time all of the malic acid had been used. This indicates a progression of heterofermentative LAB during this stage of sauerkraut fermentation rather than dominance of a single strain and/or species. Out of the 110 homofermentative LAB isolates tested for malolactic activity, only 1 was MDC$^-$. In contrast, 83 of 129 heterofermentative LAB isolates were MDC$^-$.

Identification of selected commercial LAB isolates by BIOLOG AN Microplate biochemical analysis revealed various species present during the first 10 d of sauerkraut fermentation (Table 2). Heterofermentative species included *Leuconostoc fallax*, *Leuconostoc citreum*, and *W. confusa* (also known as *Lactobacillus confusus*). Homofermentative LAB included *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus hamsteri*, and *Lactobacillus mali/sakei* subsp. *sakei*. Strain BI 119, identified by BIOLOG as *L. hamsteri* and isolated at 7 d of fermentation, did not have malolactic activity, which made it unique among homofermentors in this study. *Leuconostoc fallax*, originally isolated from sauerkraut, has been described as an acid- and ethanol-tolerant lactic acid bacterium that does not have malolactic activity (Middelhoven and Klijn 1997). Six distinct strains of *L. fallax* were recently isolated from commercial sauerkraut (7 d) that had growth and fermentation patterns very similar to *L. mesenteroides* (Barrangou and others 2002). All 6 strains were MDC$^-$. However, in this study, commercial sauerkraut isolates identified as *L. fallax* were both MDC$^+$ and MDC$^-$ (Table 2). *Lactobacillus confusus* (Harris 1991) and *Lactobacillus sake* (Kandler and Weiss 1986; Vogel and others 1993) have also previously been isolated from fermenting sauerkraut. In an effort to isolate *L. mesenteroides* strains as potential starter cultures for sauerkraut, Harris (1991) presumptively classified only 32% of the isolates as *Leuconostoc* spp. The majority of gas-producing isolates were tentatively classified as *L. confusus* (currently known as *Weissella confusa*). In the current research, we found that 15 of 17 LAB isolates randomly selected from laboratory sauerkraut fermentations (3.5 d) were gas-producing, Gram-positive cocci, and identified by the BIOLOG Microplate method as *L. mesenteroides* subsp. *mesenteroides*. All 15

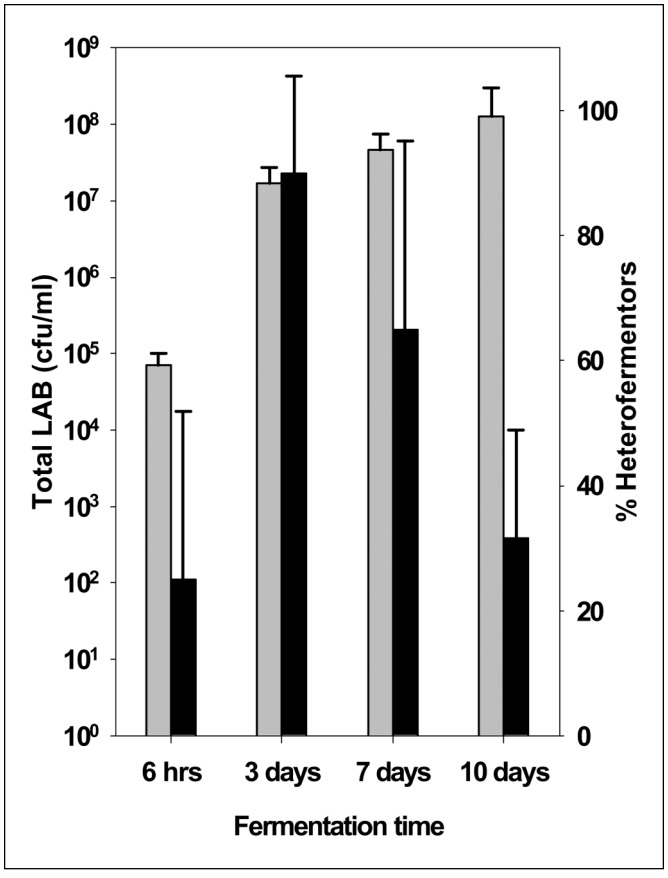

Figure 2—Lactic acid bacteria (LAB) changes during sauerkraut fermentation. Grey bars represent total number of LAB (colony-forming units [CFU]/mL); black bars represent % heterofermentative LAB; error bars represent 1 standard deviation.

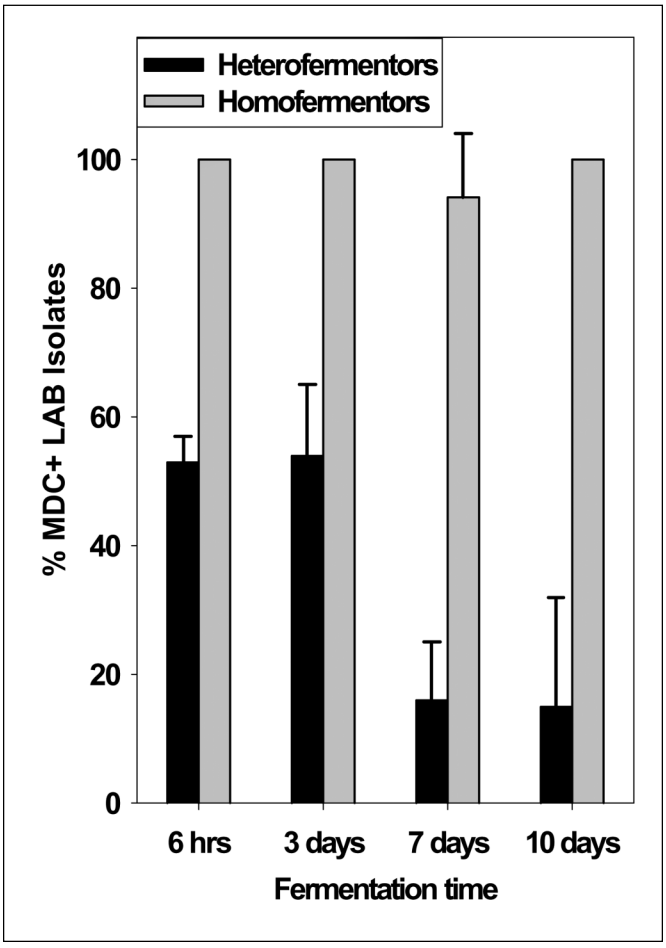

Figure 3—Malolactic activity of lactic acid bacteria (LAB) in commercial sauerkraut. Black bars = heterofermentative LAB; gray bars = homofermentative LAB; error bars = 1 standard deviation; standard deviations are zero for 6-h, 3-d, and 10-d homofermentative LAB.

Table 2—Characterization of lactic acid bacteria (LAB) from Food Fermentation Laboratory (FFL) culture collection, laboratory cabbage fermentations, and commercial sauerkraut

LAB strain[a]	Source	Time[b] (d)	Gas	Malolactic reaction (MDC)	Final pH in MRS	Identification by BIOLOG AN microplate anaerobic method
LA 81	FFL culture collection ATCC 8293 *Leuconostoc mesenteroides* type strain	NA	+	+	4.35	*Leuconostoc mesenteroides* subsp. *mesenteroides*
LA109	FFL culture collection ATCC 10881 *Weissella confusa* type strain	NA	+	+	4.43	Weissella confusa
LA10	FFL culture collection *Leuconostoc mesenteroides* strain C 33[c]	NA	+	–	4.34	*Leuconostoc mesenteroides* subsp. *mesenteroides*
LA12	FFL culture collection ATCC 23368 *Leuconostoc mesenteroides*	NA	+	–	4.36	*Leuconostoc mesenteroides* subsp. *mesenteroides*
BI 137	Laboratory brined cabbage fermentation	3.5	+	+	4.35	*Weissella confusa*
BI 111	Laboratory brined cabbage fermentation	3.5	+	+	4.49	*Weissella confusa*
BI 112	Laboratory brined cabbage fermentation	3.5	+	–	4.35	*Leuconostoc mesenteroides* subsp. *mesenteroides*
BI 127	Laboratory brined cabbage fermentation	5	+	–	4.34	*Leuconostoc mesenteroides* subsp. *mesenteroides*
BI 120	Commercial sauerkraut	0.25	+	+	4.30	*Leuconostoc citreum*
BI 129	Commercial sauerkraut	3	+	+	4.39	*Weissella confusa*
BI 109	Commercial sauerkraut	7	+	+	4.34	*Weissella confusa*
BI 117	Commercial sauerkraut	10	+	+	4.40	*Leuconostoc fallax*
BI 133	Commercial sauerkraut	0.25	+	–	4.36	*Leuconostoc fallax*
BI 123	Commercial sauerkraut	3	+	–	4.40	*Weissella confusa*
BI 132	Commercial sauerkraut	7	+	–	4.36	*Leuconostoc citreum*
BI 116	Commercial sauerkraut	10	+	–	4.36	*Leuconostoc fallax*
BI 115	Commercial sauerkraut	0.25	–	+	3.93	*Lactobacillus delbrueckii* ss *lactis*
BI 125	Commercial sauerkraut	3	–	+	4.03	No identification possible
BI 118	Commercial sauerkraut	3	–	+	4.03	No identification possible
BI 108	Commercial sauerkraut	7	–	+	4.08	No identification possible
BI 110	Commercial sauerkraut	10	–	+	4.08	*Lactobacillus mali/sakei* ss *sakei*
BI 119	Commercial sauerkraut	7	–	–	4.06	*Lactobacillus hamsteri*

[a]LA = lactic acid bacteria; BI = biological isolate.
[b]Number of days since beginning of fermentation.
[c]Received from Dr. J. R. Stamer, Dept. of Food Science, Cornell Univ.

laboratory isolates identified as *L. mesenteroides* subsp. *mesenteroides* were MDC$^-$. However, the 2 isolates identified as *W. confusa* and the *W. confusa* ATCC type strain were MDC$^+$ (Table 2). One commercial sauerkraut isolate identified by BIOLOG as *W. confusa* was MDC$^-$ (Table 2). To confirm that malolactic activity is strain dependent in *L. fallax* and *W. confusa*, identification of the isolates with another method is suggested. Some of the commercial sauerkraut LAB isolates could not be identified using the BIOLOG method, indicating the need to investigate other methods of identification. Additionally, factors such as strain variations within a species and culture conditions are known to affect sugar fermentation patterns resulting in possible misclassifications (Dellaglio and others 1995). Molecular methods (Barrangou and others 2002) may be of greater value in determining the identification of LAB. Because leuconostocs are phenotypically related to lactobacilli and pediococci and are often isolated from the same environment (Dellaglio and others 1995), it was not surprising that multiple species were present in fermenting sauerkraut. Fred and Peterson (1924) also observed a large number of bacterial types during the early stages of sauerkraut fermentation. The role of *Weissella* species in sauerkraut fermentation is unknown at this time. *Weissella confusa*, a heterofermentative LAB, was originally named *Lactobacillus confusus* because it was originally confused with *Leuconostoc* species (Kandler and Weiss 1986). In fact, the ATCC type strain for *W. confusa* was originally entered as a strain of *L. mesenteroides*. Although *L. mesenteroides* has been recognized for its importance during the initial stage of sauerkraut fermentation (Pederson and Albury 1969), it may also be valuable to recognize and investigate the role of other *Leuconostoc* species, heterofermentative lactobacilli, and *Weissella* species.

Conclusions

Malolactic activity varied among strains of *L. mesenteroides*. In commercial fermentations, the population of heterofermentors increased during the first 10 d. However, the proportion of heterofermentative LAB isolates with malolactic activity decreased, indicating that more than 1 strain or species was present during this stage. Homofermentative LAB were also present and were nearly 100% MDC$^+$. Various species of LAB, both heterofermentative and homofermentative, were identified during the first 10 d of sauerkraut fermentation, indicating that a mixture of microflora are present and may be important during sauerkraut fermentation. In cabbage juice, it was shown that the malolactic reaction occurs very early in the fermentation, both for known strains of MDC$^+$ LAB and natural sauerkraut microflora. This resulted in only a 0.1 pH unit increase at the earliest time point measured (7 h). However, sauerkraut fermentation takes place at a much slower rate than the fermentation of cabbage juice inoculated with high numbers of LAB. Because the malolactic reaction took place faster than the conversion of sugars to acids and MDC$^+$ heterofermentors were more prevalent during the first 3 d of fermentation, the malolactic reaction may play some role early in the fermentation.

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